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Award Number: DAMD17-00-1-0159

TITLE: Screening for breast cancer using near-field infrared spectroscopy of a single strand of hair

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REPORT DATE: August 2002

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command  
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;  
Distribution Unlimited

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20030319 049

# REPORT DOCUMENTATION PAGE

Form Approved  
OMB No. 074-0188

the data needed, and completing and reviewing this collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302, and to the Office of Management and Budget, Paperwork Reduction Project (0704-0188), Washington, DC 20503

1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE August 2002	3. REPORT TYPE AND DATES COVERED Annual (1 August 2001 - 31 July 2002)
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4. TITLE AND SUBTITLE  Screening for breast cancer using near-field infrared spectroscopy of a single strand of hair	5. FUNDING NUMNUMBER DAMD17-00-1-0159
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6. AUTHOR(S) Shyamsunder Erramilli, Ph.D. M. K. Hong
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7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)  Boston University Boston, Massachusetts 02215 email - <a href="mailto:shyam@bu.edu">shyam@bu.edu</a>	8. PERFORMING ORGANIZATION REPORT NUMBER
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9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)  U.S. Army Medical Research and Materiel Command Fort Detrick, Maryland 21702-5012	10. SPONSORING / MONITORING AGENCY REPORT NUMBER
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11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION / AVAILABILITY STATEMENT Approved for Public Release; Distribution Unlimited	12b. DISTRIBUTION CODE
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**13. Abstract (Maximum 200 Words) (abstract should contain no proprietary or confidential information)**

A recent Australian study has used synchrotron x-ray diffraction to identify changes in the structure of hair that may be linked to either the occurrence of breast cancer, or the increased predisposition to breast cancer because of the breast of a mutation of the BRCA1 gene. We have successfully developed a new infrared method for the detection in a single strand of hair the presence of lipid deposits that were the putative cause of the observed x-ray patterns. Our study provides an independent test of the proposed link between hair structure and breast cancer.

We have found that our table-top infrared technique does offer an alternative to expensive synchrotron x-ray sources for this purpose. However, our tests show that we find the presence of lipids in healthy patients as well. We performed independent x-ray studies in collaboration with researchers at Cornell University, who have confirmed our finding that the x-ray scattering patterns are observed in hair from healthy patients. Taken together Our work suggests that (i) IR microscopy is extremely promising, but (ii) the Australian study is likely to be wrong - a disappointing result for breast cancer patients.

14. Subject Terms (keywords previously assigned to proposal abstract or terms which apply to this award) Infrared spectroscopy; x-ray diffraction; hair	15. NUMBER OF PAGES 9
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16. PRICE CODE
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17. SECURITY CLASSIFICATION OF REPORT Unclassified Unclassified	18. SECURITY CLASSIFICATION OF THIS PAGE Unclassified Unclassified	19. SECURITY CLASSIFICATION OF ABSTRACT Unclassified	20. LIMITATION OF ABSTRACT Unlimited
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NSN 7540-01-280-5500

Standard Form 298 (Rev. 2-89)  
Prescribed by ANSI Std. Z39-18  
298-102

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## INTRODUCTION

This is an ongoing proposal for performing high resolution infrared microscopy on a single strand of human hair. This work was motivated by synchrotron x-ray scattering experiments that suggested that there are structural changes in hair samples taken from women who either have breast cancer, or have an increased genetic risk of the disease. Because infrared spectroscopy is uniquely capable of characterizing and identifying biomolecules from their spectral "fingerprints", this work has the potential for:

- (i) providing an independent test of the x-ray diffraction studies, and providing insight into the molecular basis for the structure and composition of human hair linked to the formation of breast cancer;
- (ii) leading to the development of a novel method of screening for early breast cancer based on a table-top high-resolution vibrational infrared microspectrometer.

A pilot study of 10 samples was used to test the following hypotheses : (i) Does high-resolution infrared spectroscopy have sufficient sensitivity and specificity to detect the presence of lipid domains in single strands of hair? (ii) Do the observed infrared signals correlate with x-ray diffraction data? (iii) Can IR spectroscopy of a single strand of hair be used for screening for breast cancer?

We have successfully shown that infrared spectroscopy does provide a cheap, table-top alternative method to synchrotron sources for the detection of lipid deposits. However, we find that the lipid signatures occur in hair from healthy control subjects as well. We have performed independent x-ray studies in collaboration with researchers from Cornell University. Their x-ray studies confirm that the diffuse ring, due to lipid deposits, reported by the original Australian report, is also present in healthy subjects, in agreement with our infrared studies. Taken together, this report suggests that (i) IR microspectroscopy is an extremely promising alternative table-top tool; (ii) However, the original x-ray work may have been in error, and studies on single strands of human hair will not provide a viable screening technique for breast cancer. This is disappointing.

## BODY OF REPORT

As described in our previous report, we had proposed an extension in the Task list to perform x-ray diffraction studies on the same samples that were subjected to infrared spectroscopy. This was done, at no cost to the project, because there was some disagreement in the x-ray literature:

Briefly

1) James and her collaborators first reported the correlation between a certain diffuse ring in the small angle x-ray scattering from a single strand of hair using synchrotron radiation, and the incidence of breast cancer [James et al, 1999].

2) Several separate groups published criticisms (Meyer et al, 2000; Howell et al, 2000; Briki et al, 1999), showing data that appear to contradict the original James et al result.

3) Meyer and James (2001) reported a rebuttal, affirming the essential findings of the original James et al paper, and arguing the need for following a consistent protocol in acquiring x-ray data.

4) A more recent paper (Laziri et al, 2002) reported finding no correlation between x-ray studies and the incidence of breast cancer.

There is general agreement that the diffraction ring identified by James et al (1999) does occur in hair samples, and is related to lipid deposits. The issue is whether these rings are linked to breast cancer. The relationship with lipids suggests that the original premise of our proposal – namely, that infrared microspectroscopy can offer an independent tool for studying the same structural changes – is now on very firm ground.

### Key Research Accomplishments

We outline here the research accomplishments associated with each of the approved tasks.

- Successful demonstration that infrared spectroscopy is capable of detecting the presence of lipids in hair, and therefore provides a table-top alternative to synchrotron x-ray source.
- Established that IR and x-ray diffraction studies of single strands of hair are consistent – both techniques suggest that the lipid peaks are not simply “yes-no” binary signatures, but rather form a continuum.
- Control subjects also show signatures of lipids in hair, in both IR and x-ray work, casting doubt on the original proposal of James et al (1999).

## Reportable Outcomes

### Task 1.

To obtain a high resolution infrared spectrum from a single strand of human hair.

This was accomplished successfully and spectral features characteristic of lipid deposits were identified. The same lipid deposits have been proposed as the putative source for the reported diffuse ring in synchrotron studies from the Australian group. High resolution infrared studies were performed on cryomicrotomed sections of hair, placed on Zinc Selenide windows. Spectra were collected from several thousand points within a hair sample, with attention being paid to the protein amide regions, as well the CH stretch regions near 3 microns, where lipids are known to contribute significantly. This is very encouraging, because it suggests that infrared microspectroscopy is a table-top alternative to expensive synchrotron sources.

### Task 2.

To obtain high resolution infrared spectra from single strands of human hair from a set of subjects in order to identify the presence of lipid deposits that may be correlated with increased risk of breast cancer.

In the second year of the project, this was accomplished successfully on a small sample (10) of patients from Dartmouth Hitchcock Lahey Medical Center. The review committee that commented on our first report also suggested that we take sections at several positions along the length of the hair – close to the root and further away. In nearly every sample, we found infrared signatures of lipid deposits, which was encouraging. Some of the peaks were due to aliphatic stretching frequencies, and were reported earlier. We found significant variation in the strength of the IR signatures, with no apparent correlation to the treatment protocol, or stage of disease (see Task 3 below).

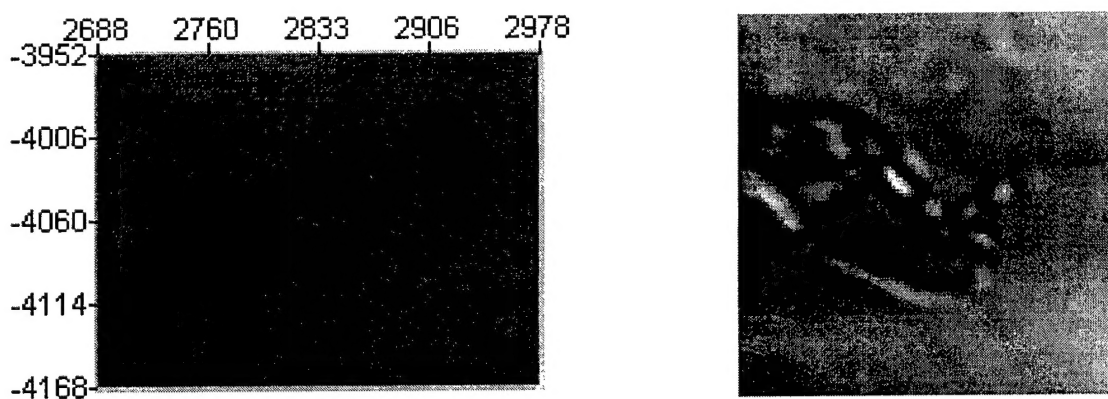
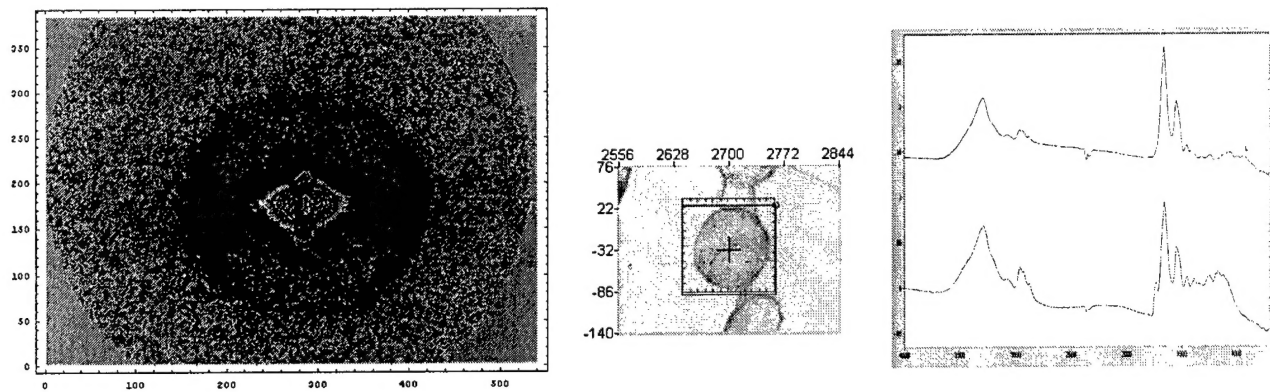


Figure: Comparison of optical image (*left*) and scanning near-field infrared (SNIM) image at  $\lambda = 10. \mu\text{m}$  of hair cross-section; near-field IR calibration standard (*right*) showing sub-wavelength spatial resolution ( $2 \mu\text{m}$ ).

**Task 3.** To perform concurrent analysis of the infrared spectra of hair obtained from cases and controls (months 7-20), in order to understand the biochemical basis for structural changes, and to test the potential of infrared microscopy as a screening tool.

Concurrent analysis was also performed on control hair samples, from healthy subjects. Again, the review committee had suggested for taking several sections along the length of the hair, close to the root and further away. We found that the same lipid signatures could be seen in the control samples as well. This was disconcerting, and was not consistent with the Australian study. So we undertook to perform x-ray diffraction studies, in collaboration with Prof Sol M. Gruner (Director, Cornell synchrotron x-ray source) and Dr Adam Martin, who agreed to work at no cost to this project. The results of the x-ray work and infrared work can be summarized as follows:

- (i) The infrared work and x-ray data obtained at Cornell are both consistent – the control samples showed the diffuse lipid ring as well.
- (ii) There was considerable variation in the intensity of the x-ray ring and in the heights of the IR lipid peaks, but the strongest signals were found in the control samples. This work suggests that the strength of the lipid peaks is not a simple “yes-no” proposition, but rather a continuous variable that can span several decades in intensity. In one patient, the x-ray peak only was discernible after overnight exposure to x-rays from a table top source. To summarize: nearly every hair sample can reveal the presence of the diffuse lipid rings. While there is considerable variation in the strengths of the peaks found, some of the strongest peaks were found in healthy patients.



*Figure 2 : Control samples show both the x-ray diffuse diffraction ring (left) and the associated infrared lipid peaks (right). Nearly every sample can show x-ray diffuse rings and lipid spectra, depending on source intensity/exposure. Lipid signatures show a continuum of intensity ranges rather than a simple binary "yes-no" correlation.*

*Task 4.* To design a portable device that can be used for rapid screening (months 20-24), dependent on the outcome of Task 3.

Because the outcome of Task 3 appears to be negative, we are not proposing to build an instrument. Rather we are proposing in the final year of the project to refine the statistics of the negative findings with a view to publishing these findings. We note that the presence of this peak in the controls, even if the number is small (10), already rules out the strong correlation proposed by the Australian group. We cannot, of course rule out a smaller correlation (given the small number of subjects studied). However, given the very high percentages required for any technique to be a useful screening tool, the studies already performed suggest that studies on a single strand of human will not be useful for breast cancer. This is disappointing.

*Personnel:* Hair samples from patients were obtained by Dr. Peter Kaufman, M.D., Dartmouth Medical College. Initial studies reported were performed by C.M. Smith, under the supervision of the Principal Investigator and Prof. M. K. Hong at Boston University. We are pleased to say that Mr. Smith has now enrolled in Medical School, and we trust that the work on this project was responsible in part in motivating him. Some of the IR work was performed by another medical student, Ji-Yeon Kim (now at Harvard Medical School) working in the laboratory at the Center for Photonics. X-ray work at Cornell is being performed by Dr. Peter Abbamonte, working with Prof. Sol Gruner (at no cost to this project).

## Conclusions

We have established that high resolution infrared microscopy is capable of acquiring infrared spectra from both lipid and protein molecules from a single strand of human hair. We have extended the studies to perform x-ray diffraction studies, in collaboration with scientists at Cornell University.

The studies have shown that (i) infrared spectroscopy does indeed provide a table-top alternative to synchrotron studies for the detection of lipid deposits in hair. (ii) there are significant lipid signatures in both infrared and x-ray from *control* samples from healthy patients.

While the number of samples studied is small (10), the observation of the lipid patterns in control samples is already sufficient to cast doubt on the original Australian proposal, that studies on a single strand of hair are not useful as a screening tool for breast cancer. This is disappointing.

Our technique works, and may provide a powerful new method for assessing biomarkers in other tissues, but the studies on hair fail – not because of a flaw in our technique, but because the original hypothesis appears to be wrong.

## Acknowledgement

This report is being filed late. The preliminary negative results were reported at the Era of Hope meeting in Orlando, and we requested feedback from other researchers and consumer advocates. Their encouraging comments and recommendations are gratefully acknowledged. We apologize for the delay, and feel that the feedback obtained was very helpful to us in planning the final year of this project.



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